THE USE OF TRANSGLUTAMINASE ENZYME IN MANUFACTURING RIPENED HARD CHEESE

By

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B.Sc. Agric. Sci. (Dairy Science), Fac. Agric., Cairo Univ., 2001

THESIS

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APPROVAL SHEET

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DEDICATION

I dedicate this work to soul of my parents and whom my heartfelt thanks: to my husband and my Prof. Dr. Mohammed Mohammed Metwally for all the support they lovely offered along the period of my study.

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ABSTRACT

Cultures were selected for their proteolytic properties to be used for Ras cheese making with transglutaminase. Cultures made contained *Lactobacillus helveticus, Lactococcus lactis* and *Lactobacillus casei* resulted in highest cheese yield, Organoleptic scores and best texture than the control of *L.bulgaricus* and *S. thermophilus*. Green tea extract could be used to stimulate lactic acid bacteria proteolysis. Green tea extract beside its activation effect, adds polyphenols which renders cheese as a functional food.

Key words: Transglutaminase, lactic acid bacteria, Ras cheese, ripened cheese , green tea extract

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INTRODUCTION

Transglutaminase is used in production of different dairy products since it improves the yield as well as the body and structure of the product, particularly, low fat products. In yogurt, the enzyme increased gel strength and decreased syneresis, particularly, in low fat yogurt, (Aprodu *et al*., 2012). In soft cheese, the enzyme gave higher gel firmness, increased the yield, decreased syneresis and improved organoleptic properties, (Mazaknait *et al*., 2013). In low fat mozzarella cheese, the enzyme increased cheese yield by increasing moisture, fat and protein recovery. The enzyme improved stretchability and meltability as well as organoleptic properties (Metwally *et al*., 2007). In low fat ice cream, the enzyme compensated for the low fat to produce a product superior in all physical and organoleptic properties than control (Metwally, 2007and Rossa *et al.*, 2011).

However, the use of the enzyme in ripened cheese was limited due to the delayed ripening process resulting from slow microbial proteolytic activity leading to a harder texture and low meltability (Özer *et al.*, 2007). This is a result of the enzyme cross-linking reaction, which reduces the availability of low molecular weight peptides used as nitrogen source by the starter (Fæaergemand *et al.*, 1999).

Aaltonen *et al*., (2014) used the enzyme for making Edam cheese from ultra-filtrated milk. Ultrafiltration of milk was used to remove the enzyme inhibitors present in milk. The enzyme was incubated with the retentate at 17˚C for 2h, then the mix was standardized with raw milk which contains the inhibitors. The resulted cheese was harder in texture

than the control. Therefore, suggested the use of peptidase to overcome the low free amino acids present in UF cheese (Aaltonen and Huumanen, 2013).

To overcome the slow activity of ripening culture in transglutaminase (MTGase) cheese, this thesis tried to select culture strains that can be active and help in normal ripening of the cheese.

Cheese slurry was used to screen microorganisms for their growth and effect on cheese ripening. Because of the lengthy ripening time required for flavor development, evaluation of each strain on quality is time consuming and costly. Therefore, cheese slurry is used to rapidly evaluate flavor and proteolytic potential of the starter in an efficient way. Therefore, this thesis was carried out in four parts.

1- Activated culture strains, single or in combinations to selecting the proper culture for their proteolytic activity using the O-Phthaldehyde method.

1-The use of cheese slurry process for selecting starter combination that can grow in TG-curd and produce enough proteolysis to help starter ripening activity.

2- The use of the selected starters for making Egyptian Ras cheese with TG-enzyme.

4- Study the effect of tea extract on the proteolytic activity of some lactic acid bacteria.

REVIEW OF LETERATURE

Microbial transglutaminase is one of the enzymes widely used for protein modification in recent years. This enzyme catalyses the formation of covalent cross-links between glutamine and lysine residues in food proteins, including milk proteins. This cross-linking reaction can modify several functional properties of milk proteins, such as solubility, heat stability, emulsifying capacity, gelation, foaming and rheological properties.

2. Sources of transglutaminase (MTGase).

MTGase is widely distributed in animal tissues and body fluids (Aeschlimann and Paulsson, 1994), plants, fishes and microorganisms (Ando *et al.*, 1989; Zhu *et al.*, 1995). Commercial MTGase was originally obtained only from animal tissues (calcium –dependent MTGase) recently, studies on the production of MTGase by micro-organisms (calcium independent)have been started. The enzyme obtained from microbial fermentation has been applied in the treatment of food of different origins. Extracellular MTGase was purified from cultural filtrate of *Streptoverticillium mobarens* from *Streptoverticillium sp.* (Ando *et al.*, 1989; from *Streptoverticillium ladakanu* and from *Streptoverticillium lydicus*. Intracellular MTGase was also found in *Bacillus subtilis* and in spherules of *physarumpoly cephalum* (Seguro *et al.*, 1995).

3. Properties of MTGase

Microbial enzyme is monomeric and simple protein with a molecular weight of about 38000 and consisting of 331 amino acids, Isoelectric point 8.9 (Jaros and Partschefeld 2006), and has a single cysteine residue and two potential glycosylation sites (Thr-Xxx-Asn-) (Yokoyama *et al.*, 2004).The optimum pH for MTGase activity was found to be between 5 and 8. However, MTGase showed some activity at pH 4 or 9 (Ando *et al.*, 1989), and was thus considered to be stable over a wide pH range. The optimum temperature for enzymatic activity was 55°C (for 10 min at pH 6.0); it maintained full activity for 10 min at 40°C, but lost activity within a few minutes at 70°C. It was active at 10°C, and retained some activity at near-freezing temperatures. MTGase is capable of gelling concentrated solutions of proteins such as soybean proteins, milk proteins, beef, pork, chicken and fish gelatin and myosins (Nonaka *et al.*, 1997; Kang *et al.*, 1994; Nielsen 1995; Seguro *et al*,. 1995; Zhu *et al*., 1995). Milk caseins, which are non-heat setting proteins, are also gelled by MTGase without heating, as is gelatin, a cold-setting protein.

3. Application of MTGase in dairy industry

a. Milk proteins and heat stability of milk

O'Sullivan *et al.*, (2001) prepared skim milk powders from control and transglutaminase-treated skimmed milk. The heat stability of reconstituted transglutaminase-treated skimmed milk (9.0% total solids) was markedly increased in the pH region of minimum stability (pH 6.8 to 7.1), compared with control milk, while the heat stability of reconstituted

concentrated transglutaminase treated skimmed milk (22.5% total solids) increased progressively as a function of pH relative to control milk.

Czernicka *et al.*, (2009) aimed to establish the best conditions of incubation of milk with the enzyme transglutaminase (MTGase) in relation to the changes of heat and ethanol stability, emulsifying and foaming properties of milk proteins. Raw and pasteurized skim milk was modified by MTGase added in the amount of 2U/g protein and incubated with the enzyme in following conditions: 40°C for 2 h, 25°C for 4 h and 5°C for 16 h. After incubation, the enzyme was inactivated by heating at 80°C for 1 min., cooled down up to 20°C and analyzed. Modification of raw milk by MTGase increased the ethanol stability, emulsifying and foaming capacity of milk proteins. The stability of formed foam was also generally better than those of control milk. In the case of pasteurized milk, modification by MTGase improved most of determined functional properties of milk proteins in comparison to functional properties of control milk. Only the foam stability of pasteurized milk after modification by TGase was not significantly higher, compared to foam stability of pasteurized control milk. The best change of functional properties of milk proteins modified by TGase was stated for the incubation conditions at 40°C for 2 h.

b. Yoghurt and acidic gel

Lorenzen *et al.*,(2002a) found that the incubation of milk with MTGase (0.05% of 1000 u/g) prior to fermentation for 2 h at 40°C, followed by inactivation for 1 min at 80°C resulted in prolonged fermentation time. The investigation of the growth behavior of yoghurt